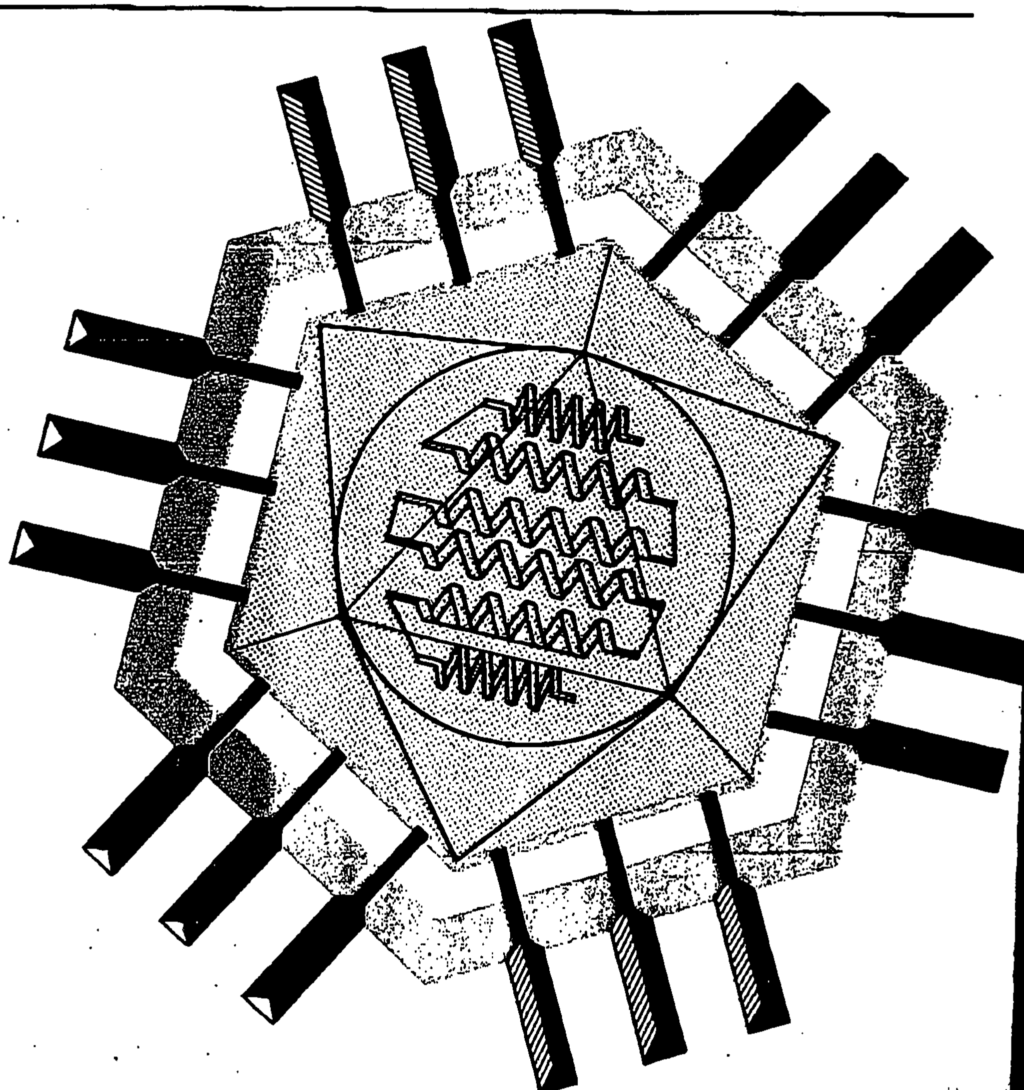
EXHIBIT A

Introduction to Modern Virology

FOURTH EDITION

N J Dimmock
S B Primrose

BASIC MICROBIOLOGY SERIES



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The assembly of enveloped viruses

A large number of viruses, particularly viruses infecting animals, have a lipid envelope as an integral part of their structure. These include many animal viruses (herpes-, toga-, oncorna-, orthomyxo-, paramyxo-, corona-, arena-, pox- and irido-), the tomato spotted wilt virus group and rhabdoviruses of plants and three families of bacteriophage (cortico-, plasma- and cystoviruses) (see Table 3.2 and Chapter 21). Herpesviruses replicate in the cell nucleus, although the viral proteins are synthesized in the cytoplasm and transported back into the nucleus. After assembly of the nucleocapsid, the virus buds off from the nuclear membrane and thus becomes enveloped. Prior to the budding process, the membrane is modified by incorporation of viral-specified proteins, which are subsequently glycosylated. Very little is known about the assembly of the lipid-containing phages and the iridoviruses, but it appears that the envelope is *not* incorporated by a budding process. In this respect, they resemble poxviruses, whose morphogenesis has been studied extensively by electron microscopy of infected cells. In thin sections, particles initially appear as crescent-shaped objects within specific areas of cytoplasm, called 'factories', and even at this stage they appear to contain the trilaminar membrane which forms the envelope. The crescents are then completed into spherical structures. DNA is added, and then the external surface undergoes a number of modifications to yield mature virions.

The majority of enveloped viruses acquire their envelope by budding from the plasma membrane or one of the internal cytoplasmic membranes (Fig. 11.13). Four events leading to the maturation have been identified. First, the nucleocapsids form in the cytoplasm. Secondly, patches of cellular membrane incorporate viral glycoproteins, which are transmembrane proteins. Thirdly, these are focused by interaction of their cytoplasmic tails with the nucleocapsid (or intermediary matrix protein), which becomes aligned along the inner surface of the modified membrane, and finally the virion is formed by budding. It is remarkable that during the budding process host membrane proteins are excluded from viral particles (retroviruses are the exception), although the bulk of the lipid in the envelope is derived from the host's normal complement of lipid.

Viruses budding into the endoplasmic reticulum are released to the exterior via the Golgi complex. First, the virus particle buds into a vesicle, which moves to and fuses with Golgi complex. Then, following the normal route of transport of cellular proteins, the virus moves through the Golgi and buds from the concave surface into another vesicle, which is transported to the plasma membrane. Fusion of the vesicle with the plasma membrane releases the particle to the exterior of the cell. Viruses which bud from the plasma membrane are automatically released when the budding process is complete.

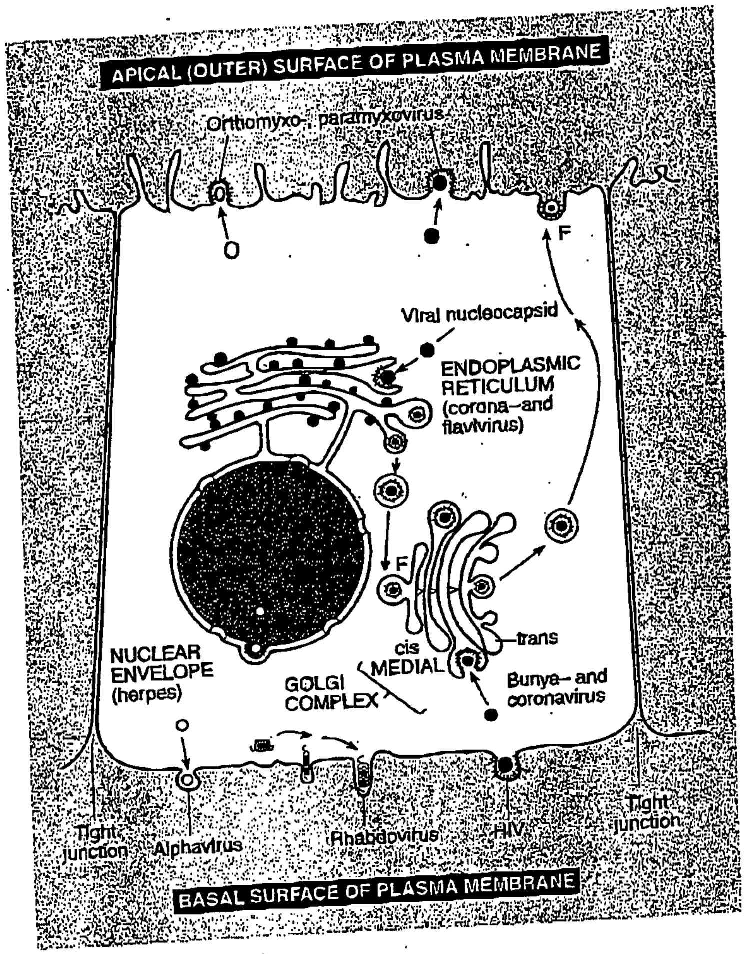

Fig. 11.13 Sites of maturation of various enveloped viruses. F, fusion of a vesicle with a membrane.

One complication is that many differentiated cells *in vivo* are polarized, meaning that they carry out different functions with their outer (apical) surface and their inner (basal) surface. For instance, cells lining kidney tubules are responsible for regulating Na^+ ion concentration. They transport Na^+ ions via their apical surface from blood to the cytoplasm and then expel them from the basal surface into urine. Some cell lines, such as Madin–Darby canine kidney (MDCK) cells, retain this property in culture, but this can only be demonstrated when they form confluent monolayers and tight junctions between cells. The latter serve to separate and define properties of the apical and basal surfaces. These properties reside in cellular proteins which have migrated directionally to one or other surface. This happens too with some viral proteins. The lipid composition of a polarized cell is also distributed asymmetrically between apical and basal surfaces. For instance, if a cell is dually infected with a rhabdovirus and an orthomyxovirus,